

### Remarks

This Amendment is being filed with a Request for Continued Examination, and a Petition for a three-month extension of time for replying to the June 28, 2005 Office Action. By the Petition and fee submitted herewith, the Applicant hereby extends the response period from September 28, 2005 to December 28, 2005. Please charge any fee deficiency, or credit any overpayment, to Deposit Account No. 50-2719.

Claims 11-14, 16-18, 20-23 and 28 are pending in the application. Claims 11 and 28 have been amended to recite that the DNA compaction agent is present at a concentration sufficient to allow the DNA insert to remain flexible. Support for amended claims 11 and 28 is found on pg. 7, paragraph [0033] of the translated specification filed on October 4, 2001. The Examiner's detailed review of the application and consideration of the Applicant's previous Response are noted with appreciation. Based on the above changes and the remarks presented below, the Applicant respectfully requests reconsideration of the claims.

### Claim Rejections – 35 U.S.C. § 102

Claims 11-14, 16-18, 22, 23 and 28 are rejected under 35 U.S.C. § 102 as allegedly anticipated by U.S. Pat. No. 6,410,220 to Hodgson et al. (Hodgson). Hodgson describes a method for directing the self assembly of gene fragments to produce a gene, gene vector or large nucleic acid molecule. As discussed in the previous Response, Hodgson adds a DNA compaction agent to the ligation reaction mix after the ligation is completed.

Here, independent claims 11 and 28 each recite the step of producing circularized recombinant nucleic acids by ligating a DNA insert and a DNA vector *in the presence of* a DNA compaction agent. The Examiner insists that Hodgson also describes the ligation of a DNA insert and a DNA vector in the presence of a DNA compaction agent, and cites to Hodgson col. 23, line 52 to support her allegation. The Applicant reiterates that Hodgson col. 23, line 52 does not specify that a DNA compaction agent is added while the ligation reaction is progressing. However, other passages of Hodgson do indicate that the DNA compaction agent is added *after* the ligation reaction has been completed. *See, e.g.*, Hodgson col. 23, line 41, which states that “[i]t is also desirable to avoid shearing the DNA once the segments *have been* joined by

ligation" (emphasis added). Hodgson discloses that one way to avoid DNA shearing is to add a DNA condensing reagent directly to the ligation reaction, and then move the DNA by pipette after it has been condensed. *See* Hodgson, col. 23, lines 49-56. One skilled in the art would recognize that the DNA would not be "moved" until the ligation reaction is completed, to ensure the greatest number of ligated molecules are formed. However, there is no disclosure in Hodgson that one must wait until the ligation reaction is completed before moving the condensed DNA. Thus, one skilled in the art would interpret Hodgson as disclosing the addition of DNA condensing reagents to the ligation reaction mixture after the ligation reaction is completed. This interpretation is further supported by Hodgson col. 24, lines 40-43, which discloses that "minichromosome DNA can be condensed . . . prior to transfection, similar to larger viral vectors." The minichromosomes are formed *in vitro* prior to condensation by ligating smaller synthetic gene fragments (see Hodgson col. 24, lines 5-6).

The Applicants respectfully point out that the phrase "ligation reaction" can refer to both the interaction between chemical compounds in a mixture (*i.e.*, active ligation), and to the state resulting from the completion of the interaction. See, e.g., the definition of "reaction" reproduced from the online dictionary "Dictionary.com":

reaction - *noun*; . . . 4(a)(1): a chemical transformation or change, the interaction of chemical entities; (2) the state resulting from such a reaction.

Moreover, claims 11 and 28 as amended recite that the DNA compaction agent is present at a concentration sufficient to allow the DNA insert to retain its flexibility. This feature also distinguishes the rejected claims from Hodgson. Because Hodgson is concerned with precipitating the DNA after ligation is complete, Hodgson provides no description of adding a DNA compaction agent to DNA in a ligation reaction that would allow the DNA to remain flexible during that reaction. To the contrary, Hodgson's compaction agent is added to the ligation reaction after ligation has been completed, in order to protect the DNA from shearing or to compact it for transforming cells. As shearing takes place when DNA strands are freely mobile in solution, Hodgson is adding compaction agent at a concentration which would immobilize the DNA; *i.e.*, cause rigidity.

Independent claims 11 and 28 recite a method that includes the step of producing circularized recombinant nucleic acid by ligating a DNA insert and a DNA vector *in the presence* of a DNA compaction agent, which DNA compaction agent is present in a concentration which allows the DNA insert to remain flexible. Because Hodgson does not disclose these features of independent claims 11 and 28, the rejection of these claims and their dependent claims 12-14, 16-18, and 22-23 under 35 U.S.C. § 102(b) should be withdrawn.

### **Claim Rejections – 35 U.S.C. § 103**

Claims 20 and 21 are rejected under 35 U.S.C. § 103 as allegedly rendered obvious by the Hodgson in view of Nagaki et al., BBRC 246: 137-141, 1998 (Nagaki).

Claims 20 and 21 depend from claim 11 and further define the concentration of compaction agent to be used according to a defined formula. Nagaki teaches that HMG 1 and 2 in concentrations of 0.05 to 2 micrograms per 10 microliter reaction mixture. Each reaction mixture contained 0.5 micrograms of linearized pUC119 plasmid – no DNA insert was included. The Office Action alleges that “it would have been *prima facie* obvious to apply Nagaki’s range of HMG concentrations with Hodgson’s method of ligating insert and vector DNA to achieve the expected advantage of achieving (sic) optimal ligation activity with a given amount of DNA and HMG.” The Applicant respectfully disagrees.

As discussed above, Hodgson teaches the addition of DNA compaction agents after the ligation reaction is completed, in order to preserve the ligated DNA from shearing during subsequent manipulations or to compact the DNA so that it can more easily be used to transform cells. There is no teaching or suggestion to add DNA compaction agent at a concentration which allows the DNA insert to retain its flexibility. On the contrary, Hodgson’s method rigidifies the ligated DNA to minimize the effect of shearing forces during subsequent manipulations or to compact the DNA sufficiently for transfection.

Likewise, Nagaki does not teach or suggest the addition of a DNA compaction agent to a ligation reaction in order to facilitate the ligation of large vector/insert combinations. Rather, Nagaki teaches that HMG1 and 2 enhance ring closure for small DNA fragments (87-123 bp), but that only circular *monomers* of pUC119 can be produced in the presence of the HMG proteins. Thus, as used by Nagaki, the ability of HMG 1 or 2 to effect ring closure strongly

decreases with increasing length – and thus flexibility - of DNA. See Nagaki, pg. 140, 1<sup>st</sup> paragraph of 2<sup>nd</sup> col., and Figs. 1-4. Moreover, Nagaki teaches that HMG-1 and 2 have “low affinity” for linear double stranded DNA, such as a linearized vector or vector insert. See Nagaki, pg. 140, 2<sup>nd</sup> paragraph of 2<sup>nd</sup> col. Thus, one skilled in the art would not be motivated to combine Hodgson with the teachings of Nagaki and use the concentrations of HMG compaction taught by Nagaki agents to form a vector with a DNA insert, as presently claimed.

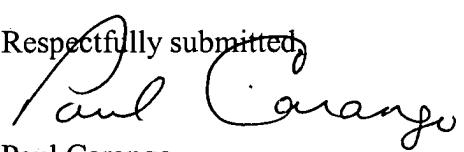
Claims 20 and 21 depend from Claim 11, which recites that the circularized recombinant nucleic acid resulting from the claimed method is greater than 5kb. The largest circularized molecule made in Nagaki is about 3 kb (*i.e.*, the size of a single circularized pUC119 vector), and HMG 1 or 2 is unable to produce circularized pUC119 dimer, trimers or greater. Nagaki also does not show that vectors greater than 5kb can be produced from linearized vector and insert DNA; in fact, Nagaki teaches away from forming such vectors by stating that HMG 1 and 2 have a low affinity for linear double stranded DNA, and by showing that circularized DNA molecules larger than about 3kb cannot be made by the Nagaki method.

The combination of Hodgson and Nagaki would not motivate one skilled in the art to practice the methods recited in claims 20 and 21, nor would one skilled in the art have a reasonable expectation from Hodgson and Nagaki that the claimed method could be successfully practiced. In fact, the cited references teach away from using DNA compaction agents in a concentration which allows the DNA insert to retain its flexibility, and from using HMG 1 and 2 proteins to produce circularized vectors with inserts. The obviousness rejection of claims 20 and 21 should therefore be withdrawn.

Conclusion

For the reasons explained above, it is respectfully requested that all of the rejections and objections set forth in the Official Action be reconsidered and withdrawn. The Applicant submits that the Application is now in condition for allowance, which is respectfully requested. If the Examiner believes that corrections as to matters of form or other minor amendments would advance the application, the Examiner is invited to telephone the Applicant's undersigned representative.

Respectfully submitted,



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